RIA-gnost® CA-50
(December 2005 - Model 12)

Kit for the radioimmunological determination of CA-50 antigen in serum or plasma.

The kit comprises :

1	vial of ^{125}**I-Anti-CA-50** (monoclonal, mouse) < 300 kBq, 22 ml buffer with bovine albumin, bovine IgG, sodium azide and a red dye.
2 x 50	**test tubes**, coated with anti-CA-50 antibodies (monoclonal, mouse).
1	vial of CA-50 **standard 0**, per 0.5 ml buffer and sodium azide.
6	vials of CA-50 **standards**, per 0.5 ml human serum and sodium azide, concentration in the nominal range of 5-180 U/ml CA-50, concentration expressed in units/ml (arbitrary system based on a reference preparation).
2	vials of CA-50 **control serum**, 0.5 ml human serum and sodium azide, concentration stated
1	vial of **incubation buffer**, 25 ml of buffer, bovine albumine, bovine IgG, sodium azide and a blue dye.
1	**wash reagent,** 5 tablets under blister.
1	plastic bag.
1	instruction for use

The dissolved reagents contain sodium azide as preservative. Avoid swallowing and contact with the skin or mucous membranes. Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

1. Introduction

Tumour cells express antigenic substances in the cell membrane which are not usually present in healthy cell membranes. The detection of these tumour-associated antigens is a valuable tool in the diagnosis of malignant disorders. Using the hybridoma technique of Köhler and Milstein, specific immunological reagents (monoclonal, antibodies, MAB) can be obtained which recognize tumour-associated antigens. A monoclonal antibody of this type, C-50 MAB, was obtained after immunization using a colorectal adenocarcinoma cell line Colo 205. The C-50 MAB recognizes two different carbohydrate chains, the sialylated Lewis-a and the hitherto unknown sialylated lactotetraose. Structures containing CA-50 are mainly found in gastro-intestinal carcinomas (e.g. pancreatic, gastric, colorectal and hepatic carcinomas) but also sometimes in other malignant growths (endometrial carcinomas). The CA-50 antigens occur in the cell membrane in a lipid-bound form (as ganglioside) and in a form bound to a higher molecular protein (as glycoprotein).
The CA-50 antigens are passed by the tumours into the blood stream where they can be specifically determined by means of immunological techniques based on C-50 MAB.

2. Clinical results obtained with RIA-gnost® CA-50

2.1. Clinical significance of the quantitative CA-50 determination

CA-50 is normally detectable only in low concentrations in the serum of healthy men and women. An increase in the CA-50 concentration can sometimes be observed in patients with benign disorders.
Pathologically raised CA-50 serum levels are encountered in the presence of CA-50 producing tumours, e.g. tumours of the pancreas, the gastrointestinal tract and the endometrium but raised CA-50 serum levels may also occur in tumours of the bladder.

2.2. Normal values

The normal range for RIA-gnost® CA-50 has been determined using serum samples from 147 healthy men and women. The statistical evaluation showed a CA-50 concentration of 19 U/ml for the 90th percentile and 25 U/ml for the 95th percentile.

2.3. Malignant disorders

If a malign disorder is suspected and elevated values for CA-50 are observed, additional diagnostic steps should be taken. The main indications for CA-50 detection are the follow up of tumour patients, i.e. the monitoring of the success of therapy, the progression or continued remission of the disease and in determining the prognosis.

2.4. Benign disorders

Benign disorders can give rise to CA-50 values which are above the normal range. The values in this group are generally slightly raised ; they include acute and chronic pancreatitis, ulcerative colitis, Crohn's disease, cirrhosis of the liver and hepatitis.

3. Principle of measurement and characteristic data of RIA-gnost® CA-50

3.1. Principle

RIA-gnost® CA-50 permits the in vitro determination of CA-50 antigen in human serum (or plasma) by the principle of a 2-step sandwich assay. During this process a complex of solid phase anti-CA-50 antibodies (monoclonal, mouse), CA-50 in the sample and ^{125}I-labelled anti-CA-50 antibodies (monoclonal, mouse) is formed. At the end of the reaction the free tracer is removed by decanting (or aspiration) and subsequent washing.
The amount of tracer bound specifically to the coated tubes is measured with a gamma counter.
Evaluation of the unknown samples is carried out by reading off from a standard curve constructed under identical conditions.
Samples outside the measuring range are diluted with assay buffer.

3.2. Imprecision
This was evaluated with 2 samples assayed 20 times in the same series and in 45 different series.

Within-run

Samples	Mean (U/ml)	CV (%)
1	16.7	3.1
2	89.2	3.4

Between-run

Samples	Mean (U/ml)	CV (%)
3	11.7	5.5
4	51.8	5.1

3.3. Detection limit
The detection limit of the method is defined as being the smallest detectable concentration different from zero. It has been determined as being 0.4 U/ml.

Note

The high sensitivity of the assay can only be achieved if the following points are borne in mind :

a) Avoid external contamination of the test tubes.
b) Ensure that the unbound tracer fraction is completely removed (decantation / aspiration). During aspiration, the capillary tubes must not become blocked ; after decantation tap them well on to cellulose.
c) Check the measuring device and any associated equipment that may be used to ensure that the background effect is kept constant, and if necessary decontaminate.
d) Exclude the adverse effect of external sources of radiation.

4. Working procedure

4.1. Equipment required
Precision micropipettes or similar with disposable plastic tips for 200 µl and 50 µl or 1 ml dispenser measuring cylinders, horizontal shaker, gamma scintillation counter.

4.2. Preparation of the reagents
The kit components, which have been stored at 2-8℃, are brought up to room temperature (17-27℃) before use. The wash buffer is prepared by dissolving the five buffer tablets in 500 ml distilled water. All unused reagents should be stored at 2-8℃. The remaining test tubes are kept in a sealed bag.

4.3. Preparation of the samples
After taking blood samples, serum or plasma is obtained by the usual methods. The serum or plasma is used directly in the assay or stored for up to three days at 2-8℃. If they are to be stored for a longer period, a temperature of –20℃ is recommended. The samples should be mixed carefully after thawing. Repeated thawing should be avoided.

4.4. Warning and precautions
Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

4.5. Assay procedure (see table)

1. A sufficient number of coated test tubes is numbered (standards, control sera, patient samples) as is given in the Table.
2. 50 µl standard (or patient's sample) are pipetted on to the base of the coated test tubes. A new pipette tip should be used for each sample.
3. 200 µl assay buffer are dispensed into each test tube.
4. The tubes are then shaken on a horizontal shaker for 2 hours at 17-27℃.
5. 1 ml wash buffer is then introduced into each test tube, decanted (aspirated) and washed with 1 ml wash buffer.
6. 200 µl ^{125}I anti-CA-50 are dispensed into each test tube.
7. The test tubes are shaken on a horizontal shaker for 2h at 17-27℃.
8. 1ml wash buffer is introduced into each test tube, decanted (aspired) and washed with 1 ml wash buffer.
9. The test tubes are then measured for 1 minute in a gamma scintillation counter.

General notes
Reagents in the same batch from more that one kit may be pooled to carry out a larger assay.

4.6. Evaluation of the results
A typical standard curve for RIA-gnost® CA-50 (coated tube) is shown in the figure 1.
The counts per minute of the individual standard S_0-S_6 are plotted against the appropriate CA-50 concentration (U/ml) on graph paper that has been prepared accordingly. The best fit standard curve is constructed through these points.
The measured values of the control sera and the patients'samples are marked on the graph and the desired CA-50 content per millilitre is read off from the standard curve.

Table : CA-50 assay procedure

	Standards (µl)							Control sera (µl)		Patients'samples (µl)		
Labelling of test tubes	S_0	S_1	S_2	S_3	S_4	S_5	S_6	C_1	C_2	1	2	Etc.
Standards S_0	50/50											
S_1		50/50										
S_2			50/50									
S_3				50/50								
S_4					50/50							
S_5						50/50						
S_6							50/50					
Control sera C1								50/50				
C2									50/50			
Patients' samples										50/50	50/50	Etc.
Assay buffer	◄---------- 200 µl ----------►											
	Shake for 2 hours											
Wash buffer	◄---------- 1 ml ----------►											
	Decant (aspirate) ; wash with 1 ml											
^{125}I anti-CA-50	◄---------- 200 µl ----------►											
	Shake for 2 hours											
Wash buffer	◄---------- 1 ml ----------►											
	Decant (aspirate) ; wash with 1 ml											
	Measure for 1 minute											

Fig. 1 Example of a standard curve

5. Radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate safety.
A summary of these is given below :
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up-to-date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone.
Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.